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Plant Meristems: What You See Is What You Get?

It remains unclear how stem cell behaviour is dynamically regulated in plant meristems. The analysis of cell behaviour immediately after silencing the *CLAVATA3* gene has now revealed specific functions in stem cell homeostasis.

Peter Doerner

The shoot apical meristem (SAM) is the ultimate source of all plant shoot cells. Continuous cell production in the meristem displaces cells towards the meristem periphery, where they organize into organ primordia. As cells exit the central zone, which contains the stem cells of the shoot, and move through the flanks to the primordia, their identity and behaviour changes: Cells in the centre have indeterminate stem cell identity, whereas cells in the periphery become determinate in the course of organ initiation. Proliferation rates are low at the centre, but increase towards its flanks and are highest in organ primordia.

Genetic analysis has identified an interacting network of genes required for meristem function in plants. This network can be summarized in a simplified model in which the *CLAVATA1–3* (*CLV1–3*) and the *WUSCHEL* (*WUS*) and *SHOOTMERISTEM-LESS* (*STM*) genes interact in a negative feedback loop to restrict stem cell numbers [1–3]. In this model, *STM* is required in the SAM to maintain the indeterminate state, whereas *WUS* is needed to maintain stem cells. *CLV3* is expressed in the central zone of the SAM in the outermost L1 and L2 cell layers (Figure 1). It encodes a small polypeptide that is delivered to the apoplastic space where it moves between cells [4]. *CLV3* encodes the presumed ligand for a heteromeric complex comprising the Clv2 and Clv1 proteins, which accumulate in the more interior L3 layer. The active Clavata complex then negatively regulates *WUS* expression by an unknown mechanism. *WUS*, which encodes a homeodomain-type transcription factor, in turn is a positive regulator of *CLV3* expression. The mechanism by which *CLV3* expression is activated is still unknown, as *WUS* is expressed in different cells than *CLV3*. This negative feedback loop insures that stem cells are restricted to the centre of the SAM and its distribution across all three layers ensures coordinated regulation of stem cell numbers throughout the central zone.

Classical analysis of mutant phenotypes has played a key role in ascribing specific function to individual genes within this interacting network. Plants defective in *CLV3* have enlarged meristems, caused by a large expansion of the central zone (Figure 1). Several mechanisms have been proposed to explain this: increased proliferation in the central zone, re-specification of peripheral cells to central cells or decreased ability of cells at the periphery to organize into organ primordia [5–7]. From the finding of low cell division rates throughout the enlarged SAM in *clv3* mutants, the latter mechanism was suggested to be the most likely [7]. However, the *clv3* morphological phenotype arises from long-term cumulative effects of the permanent loss of *CLV3* function. Such phenotypes do not distinguish between primary and knock-on, secondary effects on other genes within the interacting network or downstream of it and, therefore, may not accurately reflect the mechanistic function of individual genes.

A recent study [8] has taken a different approach to characterise gene function in interacting genetic networks. The authors developed a system to study the immediate consequences of knocking-down *CLV3* expression by inducible RNA interference (RNAi) in otherwise wild-type shoot meristems. They then examined, by confocal microscopy, rapid changes to cell behaviour in the SAM that result from removing *CLV3*. To monitor real-time behaviour of presumptive stem cells in the central zone, a *CLV3* promoter-GFP fusion gene was used, exploiting *CLV3* expression as a marker for stem cell identity in the SAM. Significantly, during all experiments in which *CLV3* was knocked down, primordia formation continued normally during the entire time course. This immediately suggested that *CLV3* does not directly function to promote cell differentiation at the periphery of the SAM to allow them to form primordia and refutes the previously favoured model for *CLAVATA* gene function.

After the *CLV3* transcript was knocked-down by RNAi, presumably causing an equivalent reduction in the abundance of the Clv3 peptide, the first changes became apparent within 24 hours

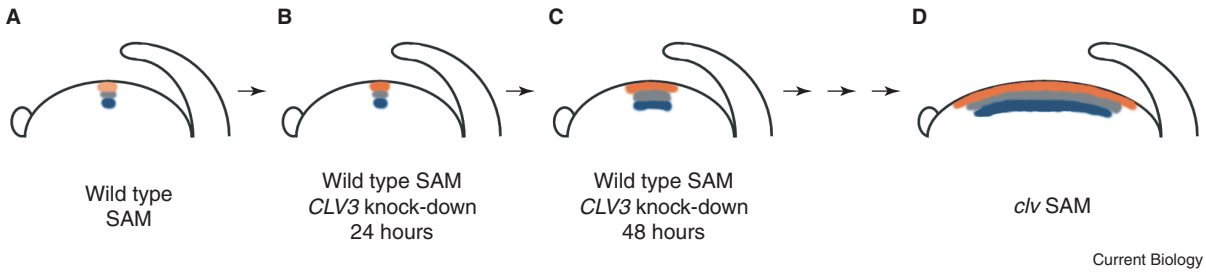

Figure 1. *CLAVATA3* controls the size of the meristem centre.

(A) In the wild-type SAM, *CLV3* expression is restricted to the outer tissue layers in the centre of the SAM (pale orange). *CLV1* (grey) is expressed in the cells below, while *WUS* (blue) is expressed in even deeper cells. (B) 24 hours after *CLV3* was silenced by RNAi, *CLV3* expression is increased (dark orange). (C) 48 hours after *CLV3* knock-down, expansion of its expression domain is observed. (D) 24 hours later, the meristem begins to expand and high proliferation is observed. Over time, cell division rates presumably decline and resemble the steady state seen in *clv3* mutant SAMs. Changes in expression patterns of *CLV1* and *WUS* in response to *CLV3* silencing are inferred.

(Figure 1B). A marked stimulation of *CLV3* expression at the meristem centre was observed, consistent with the model in which the Clv3 peptide represses the activator of *CLV3* expression, *WUS*. Within the next 24 hours, the *CLV3* expression domain began to expand, even including cells on the meristem flanks that had not divided since the start of the time course (Figure 1C). This showed that these cells could be re-specified for stem cell fate and suggested that the identity of cells transiting between the central zone and more peripheral regions of the meristem is still easily malleable. This was not entirely unexpected, as stem cell numbers normally vary to allow the SAM to adapt its activity in response to developmental or environmental signals.

After a further 24 hours, the first signs of the classical *clavata* SAM morphology emerged: the SAM began to enlarge, accompanied by a further expansion of the *CLV3* expression domain (Figure 1D). This expansion was fuelled by increased rates of cell division. This contrasts markedly with analyses performed on *clv3* mutant SAMs, where it was observed that the frequency of mitoses was uniformly low across the entire enlarged central domain [7]. It will be interesting to determine what eventually causes these lower levels of proliferation on long-term loss of *CLV3*.

It is particularly striking that enhanced proliferation was observed both outside and within the *CLV3* expression domain. The first observation implies previously unknown long-range effects of the *CLV3–WUS* network outside their 'normal' expression domain in the central zone. Is enhanced proliferation a direct effect of elevated *WUS* activity or is it caused indirectly, perhaps by increased mechanical strain arising from cell growth and division of cells within the central zone? Altered strain rates have been shown to be associated with the increased cell divisions in organ primordia [9], and altered cell wall mechanics are sufficient to stimulate proliferation in peripheral areas of the SAM [10].

The second observation strikingly showed that, even at the centre of the SAM, almost every cell had divided at least once, and many had divided more than once. This was in marked contrast to control meristems and suggests that elevated levels of proliferation are not incompatible with stem cell identity, at least transiently. However, it has been shown that *CLV3* expression might not always be a reliable marker for stem cell identity [11].

This course of events observed in shoot meristems adapting to the absence of *CLV3* would not have been predicted from the classical analysis of *clavata* shoot meristems. It will be important to follow this study up with similar studies that selectively silence *WUS* and *STM* activity. While obviously many questions remain to be answered, the report by Reddy and Meyerowitz [8] highlights the power of the new approach to analyze the inherently dynamic processes in the SAM and better understand the functions of constituent genes that interact in coupled regulatory networks. There are many such coupled regulatory networks involved in signalling in plant growth and development, for example in cell division control and in the circadian clock. It will be interesting to use this novel approach to dissect gene function in these networks as well.

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Honeybee Vision: In Good Shape for Shape Recognition

Over the past seventy years, the question of how bees learn and recognise objects has generated more controversy than consensus. New research now suggests that bees distinguish between shapes largely on the basis of their outlines.

Mandyam V. Srinivasan

The tireless lifestyle of a foraging honeybee predisposes it to learn and recognise nectar-bearing flowers quickly and accurately, so that it may return to visit them again and again. While there have been many studies documenting the ability of bees to learn and discriminate the colours, shapes and other geometrical properties of objects, we still know relatively little about how these shapes and colours are represented in the bee brain, and about how they are distinguished. A recent study by Lehrer and Campan [1] suggests that the shapes of objects are recognized in terms of the profiles of their outlines.

Imagine, for example, that bees can be trained to distinguish between a triangle and a circle of the same area. What is the basis on which they could make this discrimination? There are at least two possibilities. One is that each object is memorized and represented in the brain in a 'facet-by-facet' or 'pixel-by-pixel' fashion, rather like a digital image in a computer (see reviews [2–4]). Such a representation would, in effect, list the positions of all of the pixels that are contained within each shape, and specify the colour and intensity of each of these pixels. This would be an accurate representation of the object but, like a bitmapped image stored in a computer, it would be an expensive representation in terms of memory requirements. If objects are indeed represented in this way, then one way in which the triangle could be distinguished from the circle would be determining which representation produces the better overlap with the image that is currently being viewed, on a pixel-by-pixel basis.

Another possibility is that each object is represented largely in terms of the geometry of its outline, together with a specification of its overall colour [5]. Such a representation would be more economical in terms of memory, as it would only require specification of the positions and orientations of the edges of each object, together with some information on the object's overall colour. With this representation, the triangle could be distinguished from the circle on the basis that the outline of the former shape possesses only three orientations, while the outline of the latter shape possesses all possible orientations.

To examine this question, Lehrer and Campan [1] trained bees to distinguish between a blue square and a yellow square, by associating the blue square with a reward of sugar water. The bees learned this discrimination well. The choice preferences of the trained bees were then tested by presenting them with various pairs of stimuli. It turned out that the bees preferred a blue triangle over a green triangle, and a blue triangle over a violet triangle. Clearly, then, the bees had learnt the colour of the rewarded stimulus, namely, blue, and they were able to choose the object of the correct colour even if it had an unfamiliar shape. But had the bees also learnt the shape of the rewarded stimulus?

To investigate this, the trained bees were tested further by presenting the rewarded shape (square) together with a triangle, a diamond, or a circle. In any given test, the two stimuli in question had the same colour. This colour was blue in one group of tests, yellow in another group and black in a third group. In all of these tests, the trained bees consistently preferred the stimulus that had the correct shape (square). Thus, during the training (blue square versus yellow square), the bees had learnt not only the colour of the rewarded stimulus (blue), but also its shape (square) — although they were not being trained specifically to discriminate shapes. And in the tests they were able to choose the correct shape regardless of the colour of the object, thus suggesting (though not proving) that they were using just the outlines of the objects to analyse their shape.

Can bees, trained to distinguish between two differently shaped objects, continue to distinguish between these objects when they are of a novel colour or texture? This question was investigated in another series of experiments in which bees were trained to distinguish between a black diamond and a black circle, by rewarding them on the diamond (Figure 1). The bees learned this discrimination well. The trained bees were then subjected to a series of tests in which they were offered a choice between the diamond and the circle, presented in a range of different colours and textures. In several